

TAB 9 **Comments from the SDA/CTFA Industry Coalition
Regarding the Development of Antimicrobial Resistance
(75N-183H; C84)**

C T F

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Dockets Management Branch
Food and Drug Administration (HFA-305)
5630 Fishers Lane, Room 1061
Rockville, Maryland 20852

RE: Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Health Care Antiseptic Drug Products; Reopening of the Administrative Record; Docket No. 75N-183H

Dear Sir or Madam:

The Soap and Detergent Association and The Cosmetic, Toiletry, and Fragrance Association (Industry Coalition) hereby submit the following comments to the above referenced rulemaking. These comments supplement previous submissions that the Industry Coalition has made to the FDA on this rule-making,¹ specifically as a review of recent literature and an update in response to testimony presented at the January 22, 1997 joint meeting of the FDA's Nonprescription Drugs and Anti-Infective Advisory Committees ("Advisory Committees").

These comments are relevant to the issue of resistance that the Agency raised in the Tentative Final Monograph (TFM) that was published on June 17, 1994. The FDA states in Section 333.470 (a) (1) (iii) of the TFM that the possible development of resistance by an antimicrobial agent could be determined in two ways. One approach is the determination of the evolution of a point mutation by sequentially passing an organism through increasing concentrations of the agent. The second approach is through a survey of the published literature.

FDA has also reviewed the issue of resistance since the docket closed. At the above referenced January 22, 1997 joint meeting, FDA's Advisory Committees agreed that the evidence to date indicated that topical antimicrobial wash products do not contribute to antimicrobial resistance. They further

¹ These comments have included: comments on the TFM and the proposal of the Healthcare Continuum Model (HCCM) (June 15, 1995), compilations of efficacy data (December 13, 1995 and March 11, 1996), a detailed proposal on finished product efficacy testing methodology (September 29, 1999), a Citizen Petition for proposed labeling of HCCM categories (April 2, 2001), a Citizen Petition addressing several OTC monograph flexibility issues (June 1, 2001), a Citizen Petition on surrogate endpoint test methods (November 28, 2001), a Citizen Petition providing information in support of healthcare professional products (August 6, 2001), a Citizen Petition requesting anti-viral claims based on testing and evidence of efficacy (January 17, 2003), and a Citizen Petition providing information in support of consumer and foodhandler products (May 23, 2003). We have been advised by FDA that it is not necessary to resubmit these documents filed since the rulemaking record closed.

75N-183H **C 84**
CTFA is the national trade association representing the cosmetic, toiletry and fragrance industry. Founded in 1894, CTFA has an active membership of approximately 300 companies that manufacture or distribute the vast majority of finished personal care products marketed in the United States. CTFA also includes approximately 300 associate member companies, including manufacturers of raw materials, trade and consumer magazines, and other related industries.

The Soap and Detergent Association is the non-profit trade association representing some 120 North American manufacturers of household, industrial and institutional cleaning products; their ingredients; and finished packaging. SDA members produce more than 90% of the cleaning products marketed in the U.S.

suggested that on-going surveillance for the possible development of resistance to these agents is prudent.²

In this submission, the Industry Coalition provides a brief review of the most recent literature on topical antimicrobial ingredients that shows that

- there is evidence of decreased susceptibility of bacteria to antimicrobial agents in laboratory settings;
- there is no evidence, to date, of decreased susceptibility of bacteria to antimicrobial agents under use conditions or in the environment;
- there are reviews of the available data by other institutions that have concluded that decreased susceptibility, *i.e.*, resistance, is not a problem at the present under current use conditions; and
- there are existing surveillance programs that are available to monitor the possible emergence of resistance to topical antimicrobial agents.

Resistance to Topical Antimicrobial Ingredients

Introduction

Microorganisms resistant to drug treatments are not only the subject of numerous scientific investigations but are now commonplace items in news stories throughout the world. Few people in the developed world have not at some time in their lives owed thanks to the curative powers of antibiotics. Yet, on the other hand, the use of household and industrial biocides³ has been the subject of intense media scrutiny: do resistant organisms occur because of our reliance on, and our belief in, hygiene to keep our environment safe for ourselves and our children? Can these 'preventative' measures be the selective pressure responsible for the emergence of multi-drug resistant organisms, against which our current armory of antibiotics is failing?

Researchers have raised many questions regarding resistance. For example, does different terminology within the scientific debate lead to confusion of purpose in this area (McDonnell and Russell, 1999; Gilbert and McBain, 2003)? Another question, are the methods used to examine resistance to antibiotics and biocides equivalent and do these same methods relate to actual in-use or environmental scenarios?

Russell (2002) has raised several issues that envelop the scientific argument.

- Do antibiotic-resistant bacteria remain sensitive to biocides?
- Are biocide-resistant bacteria also resistant to antibiotics?
- Can biocides select for antibiotic-resistant bacteria?

² Transcript of the joint Nonprescription Drugs Advisory and Anti-Infective Drugs Advisory Committees on January 22, 1997.

³ Much of the literature in this document refers to "biocides", a term that includes antiseptics, disinfectants, sanitizers, topical antimicrobial ingredients, and other non-antibiotic antimicrobial agents. The focus of this submission is on topical antimicrobial ingredients.

- Can the introduction of biocides into clinical practice have an impact on antibiotic resistance?

Two further questions can be asked.

- Apart from the hospital environment, where else is it possible that the use of biocides can have an impact on the emergence of antibiotic resistant bacteria?
- What is the importance of antibiotic resistance in that scenario and its relation to others?

In this submission, the fundamentals of the resistance debate are reviewed with an emphasis on the emerging real-world data, as opposed to the controlled findings from the laboratory.

Types of Resistance

Intrinsic and Acquired

Antimicrobial resistance can either be an innate property of the microorganism or it can be acquired through transfer of external genetic material from another organism. Resistance has no single overall mechanism. Resistance to antibiotics can occur through several mechanisms, including the enzymatic modification of a target molecule, modification of the antibiotic, alteration (mutation) of the target site, or decreased access of the antibiotic to the cell interior through reduced cell wall permeability. Production of the antibiotic's target site can be up-regulated, or energy-dependent efflux pumps can eject the antibiotic from the cell (Russell *et al.*, 1997).

The cell's intrinsic resistance mechanisms are those natural properties of a microorganism that allow it, in part, to resist the actions of antibiotics and also that of biocides. The intrinsic susceptibility of an organism to an antimicrobial varies according to the nature of the antimicrobial, the microbial species, and the prevailing growth environment (Russell, 1991). Three principle forms of intrinsic resistance are associated with biocides: reduced permeability, efflux pumps, and the formation of a biofilm. In each case, the resistance mechanism effectively reduces the amount of antimicrobial reaching the interior of the cell. The growth environment of the cell can elicit a general stress response, which is an inductive adaptation to changing environmental conditions, and can result in increased resistance to physical or chemical agents (Foley *et al.*, 1999). In general, resistance to biocides has been found to be through such intrinsic mechanisms (McDonnell and Russell, 1999). On the other hand, resistance to antibiotics frequently arises from acquired mechanisms.

Mechanisms of resistance to antimicrobials obtained from external genetic material lead to the development of acquired resistance. The microorganism can acquire a plasmid (extra-chromosomal DNA capable of self replication), a transposon (chromosomal or plasmid self-inserting portions of DNA), or genetic material carried by bacteriophages. Examples of acquired resistance mechanisms include the enzymatic modification of a target molecule, modification of the antibiotic, alteration of the target site, and increased removal of the antibiotic through efflux pumps (Russell *et al.*, 1997). Many of these genetic acquisitions code for membrane efflux pumps (Levy, 2002).

McDonnell and Russell (1999) have reviewed the area of acquired resistance with respect to biocides and concluded that intrinsic mechanisms of resistance to biocides are of more importance than acquired mechanisms.

Gram-negative bacteria tend to be more resistant to antimicrobials than Gram-positive bacteria. This phenomenon is due to a second specialized membrane possessed by Gram-negative bacteria (the outer membrane) that acts as a permeability barrier to most compounds. Gram-negative bacteria also demonstrate extensive use of permeability-reducing mechanisms, including a variety of enzymatic mechanisms, which are capable of pumping out unwanted materials from within the cell. Within the Gram-negative classification, pseudomonad species are among the most recalcitrant (Kramer *et al.*, 1984; Maillard, 2002; McDonnell and Russell, 1999).

Biofilm formation results in increased resistance to disinfection as well as to antibiotic therapy (Anderson *et al.*, 1990; Vraný *et al.*, 1997). *Burkholderia cepacia* has an individually high intrinsic resistance to antimicrobials but has an even higher resistance in biofilm form. Biofilms of *Staphylococcus epidermidis* attached to Teflon® catheters can be as much as 8000 fold more resistant to antibiotics than bacteria in the planktonic state (Ramirez de Arellano *et al.*, 1994). Biofilm structure is of extreme importance as discussed in a study by Johnston and Jones (1995), which showed that when a biofilm community was dispersed, it became as susceptible to disinfection as planktonic cells. Several reports of contamination of 'disinfectant' solutions appear to have been due to inappropriate quality control resulting in the formation of a resistant biofilm phenotype during the manufacture of the product (Anderson *et al.*, 1990) or have been due to errant practice (Lee and Fialkow, 1961).

Laboratory Induced Resistance

Laboratory Strategies and Methods

The susceptibility of a microorganism to an antimicrobial is normally characterized by the minimum inhibitory concentration (MIC). MIC data are obtained using relatively standardized testing regimes (Andrews, 2001). In general, the MIC is determined by performing a two-fold serial dilution of the antimicrobial agent and testing for the growth of the microbe in each dilution. The lowest concentration of antimicrobial that prevents visible growth of the microorganism is defined as the MIC. At concentrations lower than the MIC, there is a population distribution of sensitivities towards the antimicrobial (Lambert and Pearson, 2000). The occurrence of a resistant phenotype within a population may not be obvious using this particular method.

Two fundamentally different mechanisms for obtaining a more resistant organism in the laboratory are (McBain *et al.*, 2002):

1. genotypic: genetic manipulation to introduce specific resistance genes to the microbial genome (chromosomal or plasmid borne), and
2. phenotypic: genetic selection by growth in the presence of either sub-inhibitory concentrations of antimicrobial (serial passage procedures) or supra-inhibitory concentrations of antimicrobial (direct selection procedures).

Addition of external genetic material does not necessarily confer survival advantage to an organism. Fitness to survive may only be expressed in the presence of a specific pressure, e.g., in the presence of an antimicrobial. In the absence of the specific pressure, the organism may be at a disadvantage relative to a wild-type (WT) organism (McBain *et al.*, 2002).

Obtaining a more resistant phenotype from a specific population, through passage experiments, requires careful selection of surviving organisms (capable of growth) and accurate preparation of inhibitory solutions. In essence, the distribution of intrinsic sensitivities is altered to higher MIC values, through the selection pressure of the antimicrobial agent under study. Indeed, removal of that pressure may result in the re-establishment of the WT phenotype (Russell, 2000). The resulting experiment is a time-consuming procedure, requiring multiple sub-culturing that can take several weeks to increase the MIC. However, it must also be noted that in many cases passage experiments fail to produce an adapted organism, especially with Gram-positive organisms (McDonnell and Russell, 1999).

Acquisition and Testing of Resistance in the Laboratory

Chaplin (1951) decreased the basal level of susceptibility of a bacterial culture to quaternary ammonium compounds using passage-type experiments. Using *Serratia marcescens* and careful adaptation to increasing levels of quaternary ammonium disinfectant in growth broth, the MIC was increased from 50 ppm to between 50,000 and 100,000 ppm over a period of a few days to weeks. However, the resistance was readily lost on media without the added disinfectant. Addition of lipase to the solution also resulted in a loss of resistance to just above basal level. Chaplin concluded that alterations in the lipid content were responsible for the resistance, i.e., an induced phenotypic change. Similar conclusions have been reached by Jones *et al.* (1989), Sakagami *et al.* (1989), and Nishikawa *et al.* (1979).

Although many studies have reported that the adaptive resistance is lost after sub-culture in ordinary growth medium, several recent reports have shown that passage experiments can sometimes result in a stable phenotype. Loughlin *et al.* (2002) and Joynson *et al.* (2002) both showed that *Pseudomonas aeruginosa* could be cultured, by gradual passage, to give a relatively stable, more resistant phenotype than the wild-type organism. Both groups reported that the increase in resistance observed was due to membrane changes.

Another inductive change found in Gram-negative bacteria is the overproduction of efflux pumps (McDonnell and Russell, 1999). Sub-inhibitory exposure to antimicrobial agents induces the over-expression of such pumps. Furthermore, such changes can also be caused through the acquisition of external genetic material (plasmids or transposons) or through mutation. Such mutations, or the expression of resistance genes, are also found in many Gram-positive bacteria (McBain *et al.*, 2002; Levy, 2002).

Acquired resistance to antibiotics occurs extensively, but until recently was not connected with biocide resistance with the exception of well-known resistance to certain heavy metals. With Gram-negative bacteria, McDonnell and Russell (1999) argue that there has been no unambiguous role for plasmid-specified resistance to biocides in the hospital environment. In a study where an antibiotic resistance gene from a resistant strain of *E. coli* was inserted into *P. aeruginosa*, there was no concomitant increase in the sensitivity of these strains to the disinfectants tested, as compared with the antibiotic sensitive strains of each organism (Ahonkhai and Russell, 1979). In one instance,

however, a plasmid that altered the outer membrane of *E. coli* was found to confer some resistance to quaternary ammonium compounds (McDonnell and Russell, 1999).

In contrast to Gram-negative bacteria, the insertion of an antibiotic-resistant plasmid into the Gram-positive species, *Staphylococcus aureus*, confers antibiotic resistance and elevated MIC towards disinfectants. Such plasmids contain the *qac* genes, which code for energy dependent efflux pumps capable of expelling a multitude of noxious chemicals (Levy, 2002). These *qac* genes have a high homology with those coding for antibiotic pumps (McDonnell and Russell, 1999).

The topical biocide triclosan has been at the center of a debate over transferable resistance. Some studies have claimed very high levels of resistance of *S. aureus* to triclosan (several orders of magnitude above the average MIC value of 0.01 mg/L). Although these studies have been questioned (McDonnell and Russell, 1999; Levy, 2002) because they report concentration (MIC) values far in excess of the solubility of triclosan in water, nonetheless, they continue to be published (*cf.*, Chuanchuen *et al.*, 2003). In general, the debate has focused on the comparison between methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *S. aureus* (MSSA). There is little difference with respect to triclosan susceptibility between the two (McDonnell and Russell, 1999).

Cross-resistance Studies

To what extent does resistance against one antimicrobial (or class of antimicrobial) give rise to resistance to other antimicrobials? Antibiotics tend to have specific cellular targets, whereas biocides appear to have a multiplicity of target sites, but with a site specificity that increases at lower concentrations. If these sites are the same as those attacked by a particular antibiotic, would it be possible for 'cross resistance' to occur? The debate has centered on the finding that the antibiotic isoniazid and low concentrations of triclosan both appear to target the same site in *Mycobacterium smegmatis* (McMurray *et al.*, 1999). The authors suggest that the target for triclosan is the enoyl reductase enzyme or a gene very closely linked to it. The discovery that point mutations in this gene were associated with an increase in the MIC for isoniazid (by 1.2 to 8.5 fold) and for triclosan (by 4 to 6 fold) prompted the authors to suggest that the principal target for triclosan is the enoyl reductase enzyme, although other studies provide evidence that triclosan has a number of other target sites (Suller and Russell, 2000; Lambert *et al.*, 2002; McDonnell and Pretzer, 1998). It is also important to note that the enoyl reductase enzyme is not the target site for isoniazid in the more medically relevant *Mycobacterium tuberculosis*. Mdluli *et al.* (1998) and Slayden *et al.* (2000) have shown that the primary target of isoniazid in *M. tuberculosis* is not the enoyl-ACP reductase (InhA) but rather the beta-ketoacyl-carrier protein (KasA). In addition, the KatG gene product, a catalase/peroxidase required to activate the pro-drug isoniazid in the cell, has also been identified as an additional target for isoniazid (Parikh *et al.*, 2000).

Two recent studies on biocide and antibiotic resistant *P. aeruginosa* have examined the incidence and extent of cross-resistance to other biocides and to antibiotics (Loughlin *et al.*, 2002; Joynson *et al.*, 2002). In the Loughlin study (2002), clinical isolates of *P. aeruginosa* were adapted by serial passage to be resistant to benzalkonium chloride (BAC). Their results showed that although cross-resistance to other quaternary ammonium compounds was observed, cross-resistance to other, unrelated biocides (phenolics, triclosan), was not found. Cross-resistance (increased MIC) to polymyxinB and chloramphenicol was observed with one strain, whereas increased sensitivity to the antibiotic tobramycin was found in a second strain. The authors concluded that the phenotypic

adaptation of *P. aeruginosa* to BAC does not result from a single mechanism shared by the whole species.

In a study by Joynson *et al.* (2002), four clinical isolates of *P. aeruginosa* were adapted by serial passage to exhibit increased MIC towards benzalkonium chloride (BAC) and, separately, to the antibiotics amikacin sulphate and tobramycin. In all cases the resistant phenotypes had increased generation times relative to that of the wild type (WT), which means the WT would out-compete the resistant phenotypes in a non-selective (antimicrobial-free) environment. The authors observed that BAC adapted *P. aeruginosa* had lower MIC, *i.e.*, increased susceptibility, to antibiotics than the WT, but that tobramycin- or amikacin-adapted *P. aeruginosa* demonstrated elevated MICs, *i.e.*, decreased susceptibility, towards BAC. Specifically, passage in amikacin up to 60 mg/L resulted in a shift of MIC from 0.8 mg/L (WT) to 752 mg/L, a level more than ten times the level to which the organism was originally exposed. Furthermore, amikacin adapted *P. aeruginosa* was highly resistant to tobramycin (0.4 (WT) to 133 mg/L) and tobramycin adapted *P. aeruginosa* was highly resistant to amikacin. The authors concluded that adaptive resistance to BAC and related biocides did not confer cross-resistance to antibiotics, but that adaptive antibiotic resistance conferred relatively moderate BAC resistance. Of further importance was the suggestion that exposure to sub-inhibitory concentrations of BAC could, in fact, result in an organism more sensitive towards the clinical antibiotics used.

Real-World Resistance

Speciation

Certain bacteria are less sensitive to antimicrobials than others. Some, like the pseudomonads, have a high level of intrinsic resistance to many commonly used biocides and antibiotics. However, it is important to note that this is a relative scaling. If a biocide is used at concentrations below that needed to eliminate *P. aeruginosa*, then the spectrum of the resident flora changes proportionally to the antimicrobial susceptibility of the original community. This does not mean that the biocide has selected for a resistant organism. Biofilm disinfection studies by McBain *et al.* (2003) have shown that the survivors increase in number through clonal expansion. Therefore the disinfectants have not produced a resistant organism, but they have altered the balance of the microbial community.

Stickler and Thomas (1980) showed that of 802 Gram-negative clinical isolates, 10% showed intrinsic resistance to cationic biocides. The resistant isolates were of the genera *Proteus*, *Providencia*, and *Pseudomonas*, all of which are well known for their intrinsic resistance to antibiotics. These isolates also demonstrated resistance towards common antibiotics. In contrast, the major species isolated, *E. coli* (46% of the total isolates), was uniformly sensitive to all of the agents tested. Furthermore, all isolates were sensitive to other (non-cationic) biocides such as phenolics. This work does not suggest that the use of cationic biocides resulted in the production of resistant bacteria, but that some species of micro-organisms require greater concentrations of antimicrobials to achieve the desired level of control. Finally, the authors note that the criterion used to designate whether an organism was resistant to a biocide was relatively arbitrary, unlike that used for the antibiotics.

A study by Meade (2002) suggested that triclosan-containing antibacterial liquid handwash products resulted in a detrimental ecological change in the balance of micro-organisms inhabiting a bathroom. Since *P. aeruginosa* was isolated, which because of its intrinsic resistance is more resistant to antibiotics *per se*, it was claimed that this imbalance posed a threat. However, it was also noted that the diversity of bacteria was unaltered. The basis of the work was that the "hygiene hypothesis" is valid, *i.e.*, that less disinfection and cleaning is required in order to support a natural microbiological ecology. These conclusions are in stark contrast to the voluminous work published showing that appropriate levels of hygiene, specifically targeted hygiene, has done much to reduce the incidence of disease (Bloomfield, 2002, Gilbert and McBain, 2003, Gilbert *et al.*, 2002b). The work by Loeb *et al.* (2003) also contradicts the work by Meade (2002). Loeb's work gave evidence for the importance of infection control, including increased hand-hygiene and the use of antibacterial soap. These conclusions are also found in the CDC guideline on hand-hygiene, which does much to explain the seriousness of pathogen hand-transmission in health-care settings and the need for medicated/antibacterial handwashing (Boyce and Pittet, 2002).

Problems of Phenotype and Environmental Exposure

If biofilms containing potentially pathogenic organisms are present, then antimicrobials are 'phenotypically' challenged (Gilbert *et al.*, 2002a). Such biofilms can lead to chronic infections, especially within catheters and surgical implants (Lewis, 2001). Gilbert *et al.* (2002a) argue that small changes in susceptibility towards biocides studied in planktonic culture are unimportant compared to the larger changes, which occur on establishment of biofilm. This is a view echoed by Russell (1997). Studies by Maira-Litran *et al.* (2000) have suggested that the resistance of specific *E. coli* biofilms to ciprofloxacin cannot be explained simply by *mar* mediated up-regulation of efflux. The study by Johnston and Jones (1995) showed that once a biofilm community is dispersed into solution, disinfection occurs at the same rate as for planktonic cells.

Could exposure to sub-inhibitory concentrations of antimicrobials lead to the development of a resistant phenotype in the clinical or other environment as it does in the laboratory? Others (McBain *et al.*, 2002, Thomas *et al.*, 2000) have hypothesized that downstream of the site of biocide usage, a continuum of concentration could exist, which might select for more resistant bacteria. McBain *et al.* (2002) suggest that this phenomenon has not been demonstrated in studies from "real-world" environments and that the variability of the observed links between antibiotic and biocide susceptibility suggests no single cause.

Cross-resistance studies

In the real-world (industrial, institutional, household, or clinical setting), it is difficult to demonstrate cause and effect from studies of cross-resistance. Lambert *et al.* (2001) compared the susceptibility of a range of industrial, laboratory, and clinical isolates of *P. aeruginosa*. With industrial isolates, correlations were found between the antibiotic susceptibilities, but cross-resistance to both antibiotics and biocides was not found. With the clinical isolates, this was not the case. Strong correlations were found in susceptibilities to antibiotics and certain biocides. The most recalcitrant strains were those containing aminoglycoside modifying enzymes (AME strains). Reduced cell membrane permeability was considered to be one reason why a correlation to decreased susceptibility to some biocides was found. The authors concluded that the selective pressure of antibiotic usage differentiated the clinical setting from the industrial setting. These findings are

consistent with those of Marshall *et al.* (2003), in which the home environment was considered and with those of Joynson, *et al.* (2002) considered earlier.

Triclosan is used widely to reduce skin colonization with staphylococci and has been incorporated into MRSA eradication regimes. Bamber and Neal (1999) determined the MIC of triclosan for 186 isolates of MRSA and MSSA. Fourteen isolates (7.5%) were detected where the triclosan MIC was greater than or equal to 1 ppm. There was no significant difference between the incidences of triclosan resistance in strains of MSSA and MRSA. None of 16 MRSA strains exhibiting low-level mupirocin resistance had triclosan MICs greater than 1.0 ppm. Lambert (2003a) has shown that the numbers of isolates found by Bamber and Neal with triclosan MICs greater than 1 ppm would occur from a normal population of *S. aureus*. Therefore, there is no evidence that the MICs observed by Bamber and Neal are other than that expected from a normal population (strain) distribution.

A study by Suller and Russell (2000) showed that a triclosan sensitive strain of *S. aureus*, a more resistant MRSA strain, and a triclosan resistant mutant exhibited almost identical rates of disinfection with triclosan, even though the MIC to triclosan of the resistant strains was 40 times higher than that of the sensitive strain. With a single target, increased MIC towards an antibiotic can be related to decreased bactericidal activity, but the same is not true for biocides since they have a multiplicity of target sites. This paper and the one by Lambert *et al.* (2002) help show that triclosan has multiple target sites of antimicrobial action.

A report by Lambert *et al.* (2002) on the MIC of eight biocides and several clinically relevant antibiotics for 256 clinical isolates of *S. aureus* (169 MSSA, 87 MRSA) showed no significant increases in the mean population MIC for MRSA or MSSA strains isolated between the years 1989 and 2000. Although many MRSA isolates demonstrated elevated MICs toward numerous antibiotics, there was no difference in the mean population MIC of triclosan between MRSA and MSSA. These data agree with those of Bamber and Neal (1999). A similar analysis of 111 clinical isolates of *P. aeruginosa* showed significant decreases in the mean MICs of several antimicrobials including antibiotics over the same time period (Lambert *et al.*, 2002). Suller and Russell (2000) have also demonstrated in laboratory studies that *S. aureus* resistance to triclosan does not lead to antibiotic resistance. In their study, cross-resistance to the antibiotic mupirocin was not achieved. Mupirocin resistant *S. aureus* that were also more resistant to triclosan have been reported. These isolates showed no cross-resistance to other biocides such as quaternary ammonium compounds (Cookson *et al.*, 1991).

Using these data, Lambert *et al.* (2002) described numerous correlations between the MICs of antibiotics and biocides. However, many of these correlations were negative, *i.e.*, an increase in MIC of a particular biocide was correlated with a decrease in the mean MIC of a particular antibiotic. Advanced statistical investigation using the method of principal component analysis grouped, in general, the antibiotics and the biocides separately. The groupings appeared to reflect the mode of action of the antimicrobials. In many cases the groupings showed little interaction, suggesting that little cross-resistance exists between the different groups.

Rutala *et al.* (1997) examined hospital strains of antibiotic resistant bacteria for altered susceptibility to disinfectants. In a series of comparative trials with multiple replicates, in only one case was an organism designated as antibiotic resistant (a strain of *Klebsiella pneumoniae*) found with a raised MIC to a disinfectant. In three cases antibiotic resistant strains were more susceptible to the

disinfectants than the corresponding antibiotic sensitive strains. It should be noted that the disinfectants used were combinations or blends of materials. The 'phenolic' disinfectant was a combination of at least four active materials, and the quaternary ammonium disinfectant was a mixture of several types of surfactants with varying sizes of hydrophobic tail. For resistance to occur, the cell would have to, presumably, become resistant to all components of the mixture at the same time.

Home environment

A study by Marshall *et al.* (2003) compared the incidence of bacteria, including antibiotic resistant bacteria, in the homes of users and non-users of antibacterial agents. The authors concluded that high frequencies of antibiotic-resistant bacteria occurred in the home environment in both groups. However, there were no significant differences in the overall titers of bacteria, potential pathogens, or frequencies of antibiotic resistance in a single-time analysis of homes whether using or not using antibacterial-containing products. This latter finding echoes the work of Josephson *et al.* (1997) who also showed that significant reduction in numbers of bacteria occurred when study participants were instructed in the proper use of biocides.

In a similar study Cole *et al.* (2003) sampled 60 homes split evenly between users and non-users of biocides. Four common household biocides were selected for this study: triclosan, p-chloro-m-xyleneol (PCMX), pine-oil, and a quaternary ammonium surfactant. As in the Marshall study (2003), there was no significant difference found in the level of antibiotic resistance between the users and non-users. The results also showed no evidence of cross-resistance between antibiotics and biocides in either the users or non-users. The non-user group did, however, have a significantly greater number of potential pathogenic organisms present.

Hospital Environment

Of relevance to this discussion is a study that concerns an MRSA outbreak and links the hospital environment with the home (Masterton *et al.*, 1995). Although normal amelioration strategies were enforced within the hospital environment, eradication of the MRSA was not successful. A nurse was found to be a carrier of the organism; she repeatedly inoculated herself in her home environment, to which she had transferred the organism from the hospital. The outbreak ended after improved hygiene measures were instituted in the nurse's home environment.

The CDC guideline for hand hygiene in health-care settings does much to explain the seriousness of pathogen hand-transmission in health-care settings and the need for medicated/antibacterial handwashing (Boyce and Pittet, 2002). This need is exemplified in a report of a large hospital outbreak of antibiotic resistant *Acinetobacter anitratus*, isolated from catheters, presumably present in biofilm form (French *et al.*, 1980). Prompt identification in conjunction with stricter hand-washing and disinfection regimes led to the successful control of the organism.

Marshall *et al.* (1997) reported the results of an intensive program of antiseptic hand-washing, with a triclosan-based medicated soap, aimed at combating an MRSA infection episode. Not only did the incidence of MRSA decrease significantly, but the percentage of ciprofloxacin-sensitive isolates increased from 8.1% to 22.5% within the trial.

The Hospital Infection Control Practices Advisory Committee (HICPAC, 1995) recommended the use of disinfectants for environmental cleaning to reduce the spread of vancomycin resistant *Enterococcus faecium* (VRE). Saurina *et al.* (1997) examined the activity of disinfectants against VRE and concluded that alcohol, bleach, phenolic, and quaternary ammonium based disinfectants worked effectively. They also stated that a hydrogen-peroxide based disinfectant was not appropriate for use in the hospital, concluding that it was important, as part of an infection control strategy, to verify the activity of the disinfectant in actual in-house conditions. Penna *et al.* (2001) found similar results. This latter point is important because the recommended in-use concentrations given by the manufacturer may be appropriate for the vast majority of uses but may have reduced efficacy under certain conditions. Furthermore, the efficacy of disinfectants under varying conditions (Lambert, 2003b), and the selection criteria and use of cleaning and disinfecting agents have recently been reviewed (Sandle, 2003).

Poultry Environment

Antibiotic resistant, plasmid bearing *Listeria monocytogenes* strains isolated from poultry products were examined for their ability to resist disinfection. No significant difference was found between the isolates with or without the plasmid with respect to biocide susceptibility. The persistence of these plasmid containing strains within the poultry processing factory could not be attributed to the use of the disinfectants (Earnshaw and Lawrence, 1998).

Industrial Environment

Lear *et al.* (2002) examined over 100 triclosan and PCMX factory isolates and compared their MICs for triclosan and chloroxylenol to those of the equivalent culture collection strains. They concluded that there was no evidence that the residual levels of biocides in the factory environment had led to changes in susceptibility. Equally, a study (Braid and Wale, 2002) of triclosan-impregnated storage boxes showed that the antimicrobial was effective at reducing the numbers of various challenge inocula and that susceptibility of the strains was unaffected after repeated exposure on these treated items.

Gilbert and McBain (2003) have recently reviewed much of the literature concerning studies of cross-resistance to biocides and antibiotics in the workplace. They concluded that "field studies in environments where biocide use has been high failed to demonstrate the evolution and selection of biocide and antibiotic resistant-clones, rather they demonstrate a clonal expansion of pre-existing, resistant but less competitive species."

Real-world Conclusions:

In the real-world (industrial, institutional, household, or clinical setting), cause and effect from studies of cross-resistance do not clearly link antibiotic resistance with resistance to other antimicrobial agents (Lambert *et al.*, 2001). Resistance to antibiotics generally arises at one target or through one metabolic pathway and confers absolute resistance on the organism, which does not respond to the antibiotic. On the other hand, resistance to biocides is often relative; allowing a higher use concentration to kill the resistant organism. Furthermore, topical antimicrobial ingredients generally act at multiple sites/metabolic pathways in a microorganism. The generation of absolute resistance to antimicrobial agents would require multiple intrinsic and/or acquired changes

to a microorganism, one that would not compete in the environment when the antimicrobial was withdrawn.

The Need for Vigilance and Action

In 1997, the FDA's Advisory Committees met to review the issue of potential development of cross-resistance to antibiotics due to the use of topical antimicrobial ingredients. The opinion of the panel was that the evidence to date indicated that topical antimicrobial wash products did not contribute to the development of antimicrobial resistance. They further suggested that on-going surveillance for the possible development of resistance to these agents was prudent.

In 1998, the British House of Lords Select Committee on Science and Technology published a report on *Resistance to antibiotics and other antimicrobial agents*. It called for a restriction on the inappropriate use of antibiotics, which they stated encouraged resistance, and for improved infection control and basic hygiene across the United Kingdom's National Health Service (House of Lords, 1998; House of Lords, 2001).

There were also calls for improved surveillance of possible problem areas. The British National Health Service has implemented the UK Antimicrobial Resistance Strategy and Action Plan, which has three main themes: surveillance, prudent antimicrobial use, and infection control (Department of Health, 2000). Other governmental and global organizations have published similar findings and strategies (Ministries of Health, Food, Agriculture, and Fisheries, 1999; National Board of Health and Welfare, 2001; WHO, 2000; WHO, 2001; WHO, 2002).

Within the European Commission's Health and Consumer Protection Directorate-General the Scientific Steering Committee has recently published its findings on triclosan resistance (European Commission, 2002). They found that although "sound scientific laboratory evidence exists for the development of Triclosan related mechanisms for antimicrobial resistance, ... the evidence as to whether these mechanisms are shared by other antimicrobial agents or whether they are transferable to micro-organisms other than those used in the laboratory is limited and contradictory."

Furthermore they state, "No evidence of such resistance has been seen so far in clinical isolates, and there is no epidemiological evidence to suggest a problem in clinical practice. There are, however, very few targeted studies of resistance to Triclosan in relevant clinical or wider environments."

They conclude that "Triclosan is a useful and effective biocide which has been safely used for many years across a broad range of dental, medical, cosmetic and household products and is increasingly finding a use in clinically important applications. There is no convincing evidence that Triclosan poses a risk to humans or to the environment by inducing or transmitting antibacterial resistance under current conditions of use."

The U.S. has existing programs that can be used to track antimicrobial resistance. These include the National Nosocomial Infections Surveillance (NNIS) program and the Interagency Task Force on Antimicrobial Resistance (NNIS, 2002; CDC, 2003). The NNIS is a joint program that has been in place since 1970 between the Centers for Disease Control and Prevention (CDC) and 315 acute care hospitals. This program can track trends in antimicrobial resistance. A more recent program is the Interagency Task Force on Antimicrobial Resistance that is a joint program between the CDC, the National Institutes of Health, and the Food and Drug Administration together with other affiliated

agencies and departments. This task force is developing and implementing a coordinated national plan for the surveillance of antimicrobial resistance (CDC 2003).

Concluding Remarks

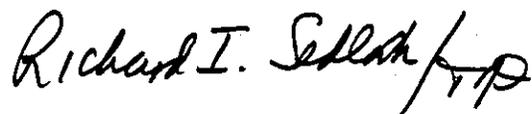
This submission addresses several questions regarding the link between antimicrobial usage and antibiotic resistance. In general, antibiotic resistant and antibiotic sensitive bacteria are equally sensitive to the *in-use* concentration of antimicrobials (Russell, 2000). In some cases a decreased susceptibility to an antimicrobial is linked to a decrease in susceptibility to an antibiotic. However, there are also examples where decreased susceptibility to an antimicrobial can increase the susceptibility to an antibiotic. In other cases there is no such correlation proven, *e.g.*, populations of MRSA are as equally sensitive to triclosan as are MSSA. There is no evidence in real world situations outside the laboratory that antimicrobials can select for antibiotic resistant bacteria. If the use of antimicrobials has an impact on antibiotic resistance, then the effect is believed to be very small (Gilbert and McBain, 2003). Other environments in which it could be speculated that antimicrobials could have an impact on the emergence of antibiotic resistance are the home, food, and industrial environments, but there is no evidence to date of such an impact.

Finally, this submission has shown that, whereas in the laboratory environment, there is evidence of decreased susceptibility of bacteria to antimicrobial agents, in the real world environment, there is no evidence of decreased susceptibility of bacteria to antimicrobial agents. In recent years, several international governmental and non-governmental agencies (British House of Lords, European Commission, WHO, and others) have reviewed the available data on antimicrobial resistance. None have identified resistance associated with the use of topical antimicrobial products as a concern under current conditions of use. Additionally, an independent review of the available resistance data has come to the same conclusion (Goodfellow *et al.*, 2003). The FDA's own panel found that resistance associated with the use of topical antimicrobials was not a problem at that time under the contemporary conditions of use. In order to ensure that resistance does not become a problem in the future, it would be prudent to continue surveillance for its development. There are existing surveillance programs that are available to monitor the possible emergence of resistance to topical antimicrobial agents. These include the National Nosocomial Infections Surveillance (NNIS) and the Interagency Task Force for Antimicrobial Resistance (CDC 2003).

Sincerely,



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